Chinese Science Bulletin

Cellulose-hydrogen production from corn stalk biomass by anaerobic fermentation

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Cellulose-hydrogen production from corn stalk by lesser panda manure was carried out in batch tests and a 5 L scale-up continuously stirred anaerobic bioreactor (CSABR), respectively. The bio-pretreatment of corn stalk was found most effective at 25℃ using microbe additive of 7.5 g/kg, in which the yields of soluble saccharides (SS) and lactic acid were 212 mg/g-TS and 21 mg/g-TS, respectively. The maximum cumulative H_2 yield (176 ml/g-TS) and H_2 production rate (14.5 ml/g-TS h^{-1}) were obtained at pH 5.5, 36℃ by treating a substrate of 15 g/L. The hydrogen content in biogas was 57.2% and there was no significant methane gas observed. During the optimal period of H_2 production, the ORP values stayed in the lower level ranging from –445 mV to –455 mV. The results show that the bio-pretreatment of the raw materials played a vital role in the effective conversion of corn stalk into cellulose–hydrogen by mixed culture.

lesser panda manure, corn stalk, bio-pretreatment, cellulose-hydrogen, anaerobic fermentation

Nowadays, global energy requirements are mostly dependent on fossil fuels, which eventually lead to a serious threat to our economy, environment, and energy security. In recent years, a great deal of attention is being paid to the utilization of bio-ethanol from grain as alternative and eco-friendly fuel throughout the world, which would lead to food shortage and price rise of grain. Thus, there is a pressing need to develop non-polluting and renewable energy source.

Compared with other fuels, hydrogen has the advantages of being renewable, providing high calorific value of 142.35 kJ/g and producing only water upon combustion. In addition, it can also be used for the syntheses of ammonia and the hydrogenation of edible oil, petroleum and coal. People believe that hydrogen-based economy may contribute to meeting the growing worldwide demand for energy.

Traditionally, hydrogen is mainly produced by hydrocarbon reformation and electrolysis of water, which are energy-intensive, unfriendly to environment, and still dependent on fossil fuel. From the aspect of energy security and environmental protection, bio-hydrogen production has been an exciting area of bio-energy production. At present, however, most studies on the production of bio-hydrogen have been confined to treating pure carbohydrates and carbohydrate-rich wastewater[1–4].

An alternative of bio-hydrogen production is to convert cellulosic biomass (e.g., corn stalk) to cellulose-hydrogen as a high value-added product, since cellulosic biomass is a valuable and most abundant renewable resource on the earth. The annual yield of natural cellulosic biomass in China alone exceeds 0.7 billion tons, in which the amount of corn stalk is around 220 million tons. So far, however, a few cases have been reported for cellulose-hydrogen production from biomass because of their complex chemical structures. For instance, the maximum H_2 yields of treating raw corn stalk and wheat

Received July 15, 2008; accepted December 17, 2008
doi: 10.1007/s11434-009-0147-x
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Supported by the National Basic Research Program of China (Grant Nos. 2009CB220005 and 2006CB708407), the National Natural Science Foundation of China (Grant Nos. 90610001 and 20871106) and Energy and Technology Program from Zhengzhou University

Citation: Xing Y, Ma H C, Fan Y T, et al. Cellulose-hydrogen production from corn stalk biomass by anaerobic fermentation. Chinese Sci Bull, 2009, 54(8): 1434-1441, doi: 10.1007/s11434-009-0147-x

straw were only 2.68 mL/g and 0.5 mL/g, respectively[5]. To enhance the hydrogen production potential of biomass, the substrate was generally pretreated by chemical/physical methods. Fan et al. reported that the H_2 yields of corn stalk, beer lees and wheat straw pretreated by dilute acid were 126.9 mL/g, 54.4 mL/g and 68.1 mL/g, respectively[6–11]. However, no information is available on the cellulose-hydrogen production using bio-pretreated corn stalk.

In this context, the objective of this study was to investigate the effects of the H_2-producing micro-flora and pretreatment method on cellulose-hydrogen production from corn stalk. The scale-up test was successfully carried out in a 5 L continuously stirred anaerobic bioreactor (CSABR).

1 Materials and methods

1.1 Seed micro-flora

The natural microbial consortium, lesser panda manure and cattle manure, were obtained from the Zhengzhou Zoo and the Dairy Center of Zhengzhou City, respectively. The two kinds of micro-flora were continuously aerated by forced-air pumping for 24 h at ambient temperature, respectively, so as to inhibit the bioactivity of H_2 consumers and harvest high yield H_2-producing spore-forming anaerobes, and then were pre-incubated with glucose in anaerobic reactors at 36℃ for 2—3 days, respectively.

1.2 Experimental materials

Fresh-cut corn stalk at harvest maturity used as the substrate was collected from a single field in the suburb of Zhengzhou City, and then ground by a vegetation disintegrator (FZ102) to pass 40-mesh screen. Its characteristics were as follows: total solid (TS, the matter that remains as residue upon evaporation and drying at 103℃ to 105℃) 75%, cellulose 33.64%, hemicellulose 24.4%, lignin 8.65% and soluble saccharides(SS) 0.073 g/g-TS. The liquid cellulase (enzyme activity 2.2 IU/mL) was provided by the Ningxia Heshibi Co., one unit of enzyme was defined as the amount of enzyme capable of producing 1μmol of reducing sugars in 1 min.

The microbe additive was provided by Zhongliang Co., mainly composed of compound bacillus ($\geqslant 2.0\times10^{10}$ bacteria/g), lactobacillus plantarum ($\geqslant 5.0\times10^{9}$ bacteria/g) and saccharomycete ($\geqslant 6.0\times10^{9}$ bacteria/g).

Each liter of nutrient stock solution contained 80 g of NH_4HCO_3, 12.4 g of KH_2PO_4, 0.1 g of $MgSO_4\cdot7H_2O$, 0.01 g of NaCl, 0.0278 g of $FeCl_2$, 0.01 g of $Na_2MoO_4\cdot2H_2O$, 0.01 g of $CaCl_2\cdot2H_2O$, and 0.015 g of $MnSO_4\cdot7H_2O$.

1.3 Pretreatment of the substrate

Acid pretreatment: the ground corn stalk was pretreated at 120℃±1℃ for 1h with dilute acid at solid loading of 10% (*w*/*w*), and then neutralized to pH = 7.0 with dilute NaOH solution.

Acid-enzyme coupling pretreatment: the mixtures of the acid pretreated corn stalk and the liquid cellulose (solid loading of 5% (*w*/*w*)) were sealed in serum bottles, and operated at 50℃±1℃ for 72 h with a rotation speed of 90 rpm.

Solid bio-pretreatment: the mixtures of the ground corn stalk and the microbe additive were sealed in serum bottles, and reacted under anaerobic condition at 25℃ for 15 days. Meanwhile, the pH value of medium was not further adjusted.

1.4 Experimental procedure

The batch experiments were performed with 150mL serum vials, each was filled with 50 mL mixture comprising the pretreated corn stalk, 2 mL of nutrient stock solution, and 30 mL of pre-incubated inoculums. The pH of mixtures was adjusted to 7.0 before cellulose-hydrogen fermentation. These vials were filled with nitrogen gas to remove oxygen and create an anaerobic environment for the cellulose-hydrogen production, and then capped with rubber stoppers. The bottles were placed in an orbital shaker at 35℃±1℃ and operated with a rotation speed of 120 $r\cdot min^{-1}$. All the experiments were carried out independently in triplicate.

1.5 Analytical methods

The biogas volume at regular intervals was measured by releasing the pressure in the bottles using displacement of saturated brine. The concentrations of H_2, CO_2, CH_4, VFAs, and ethanol were analyzed by Gas Chromatography (GC, Agilent 4890)[1]. The SS and lactic acid were determined by the methods of P-hydroxydiphenyl colorimetry and DNS using HP 8453 UV-Visible system, respectively[12].

1.6 Data analysis

Hydrogen gas yield was calculated from the headspace

measurement of gas composition and the total volume of biogas produced at each time interval using the mass balance equation:

$$V = V_0\gamma_i + \sum V_i\gamma_i, \quad (1)$$

where V is the cumulative H_2 gas volumes at the current; V_0 is the volume of headspace of vials; V_i is the biogas volume discharged from the vials at the time interval (i); γ_i is the fraction of H_2 gas discharged from the vials at the time interval (i).

The cumulative volume of H_2 production in the batch experiments followed the modified Gompertz equation:

$$H = P\exp\left\{-\exp\left[\frac{R_m e}{P}(\lambda - t) + 1\right]\right\}, \quad (2)$$

where H is the cumulative H_2 production (mL), λ is the lag time (h), P is the H_2 production potential (mL), R_m is the maximum H_2 production rate (mL/h), and e is 2.718281828. The values of P, R_m and λ for each batch were estimated using the solver function in Excel with a Newtonian algorithm[13]. In this study, P_s (defined as mL/g-TS) and R'_m (expressed as mL/g-TS·h^{-1}) were calculated by dividing P and R_m by the initial TS content of the substrate, respectively.

2 Results and discussion

2.1 Effects of H_2-producing micro-flora on hydrogen production potential

Table 1 summarizes the estimated kinetic parameters from 0.1% lactic acid pretreated corn stalk (15 g/L) by cattle manure and lesser panda manure at initial pH 7.0 and 36℃, respectively. The R_2 values of empirical models for cattle manure and lesser panda manure were 0.989 and 0.996, respectively. The high values of regression coefficient suggest that the empirical models fit well to the actual data. As can be seen from Table 1, the values of R'_m and P_s sharply increase from 4.6 mL/g-TS·h^{-1} and 89.2 mL/g-TS by cattle manure to 9.1 mL/g-TS·h^{-1} and 109 mL/g-TS. It indicates that the H_2-producing microflora have a significant influence on the bio-hydrogen production[8].

Table 1 Kinetic parameters in the modified Gompertz Equation

H_2-producing micro-flora	Substrate concentration (g/L)	Initial pH	λ(h)	R'_m (mL/(g-TS·h))	P_s (mL/(g-TS))	R^2
cattle manure	15	7.0	11.7	4.6	89.2	0.989
lesser panda manure	15	7.0	11.5	9.1	109	0.996

The H_2-producing characteristic of lesser panda was further investigated (Table 2). The results show that the glucose, maltose, lactose, xylose, sucrose, cellobiose, and microcrystalline cellulose were the preferred substrates for the hydrogen production by lesser panda manure in the pH range of 5.0—7.0. The H_2 yield increased rapidly from 134 mL/g-substrate to 298 mL/g-substrate. The maximal H_2 yield of 298 mL/g-substrate was obtained by treating glucose of 5 g/L at initial pH 6.5. In addition, the microcrystalline cellulose was also degraded to produce H_2 (67 mL/g). The result was not consistent with the previous reports, it is generally believed that the large molecules polymer can not be degraded by the pure H_2-producing strain[14,15]. Based on above results, the pre-incubated lesser panda manure was employed as H_2-producing micro-flora in the batch tests.

Table 2 Kinetic parameters for hydrogen production process from different substrates

Carbon source	P_s (mL/g)	R'_m (mL/(g·h))	λ (h)	R^2
glucose	298	30.3	3	0.9936
maltose	243	24.6	4.5	0.9991
lactose	134	11.4	16.5	0.9922
xylose	189	13.8	4.5	0.9956
sucrose	275	24.8	2	0.9916
cellobiose	257	30.8	4.5	0.9956
microcrystalline cellulose	67	4.1	13	0.9894

2.2 Effects of substrate pretreatment on hydrogen production

It is well known that the direct conversion of natural cellulose biomass by H_2-producing bacteria is considerably hard because of its complicated polymer structure, such as cellulose, hemi-cellulose, and lignin. For instance, the maximum H_2 yield of raw corn stalk was only 20 mL/g-TS by pre-incubated lesser panda manure. To enhance the hydrogen production potential of corn stalk, the influences of substrate pretreatments on the yields of soluble saccharides (SS) and H_2 were further discussed at substrate concentration of 15 g/L and initial pH 7.0, respectively.

(i) Dilute acid pretreatment. Figure 1 illustrates that the yields of SS and H_2 were significantly dependent on the acid types and acid concentrations. For instance, the SS yield increased with lactic acid concentration from 0.04% to 1.0%. The maximum SS yield of 212 mg/g-TS was observed at the lactic acid concentration of 0.4%. After the pretreatment by HCl/H_2SO_4, the SS yields in-

creased remarkably from 166 mg/g-TS and 172 mg/g-TS to 343 mg/g-TS and 350 mg/g-TS in the range of HCl 0.04% to 1.0%, and H_2SO_4 0.6% to 2.0%, respectively, then decreased slightly with the further increase of HCl or H_2SO_4 (4.0% HCl, 309 mg/g-TS; 3.0% H_2SO_4, 341.6 mg/g-TS). The phenomenon revealed that higher concentration induced the increase in the generation of metabolic by-products, such as 5-hydroxymethyl furfural, levulinic acid, phenolic compounds, and resulted in the decrease of SS[16,17].

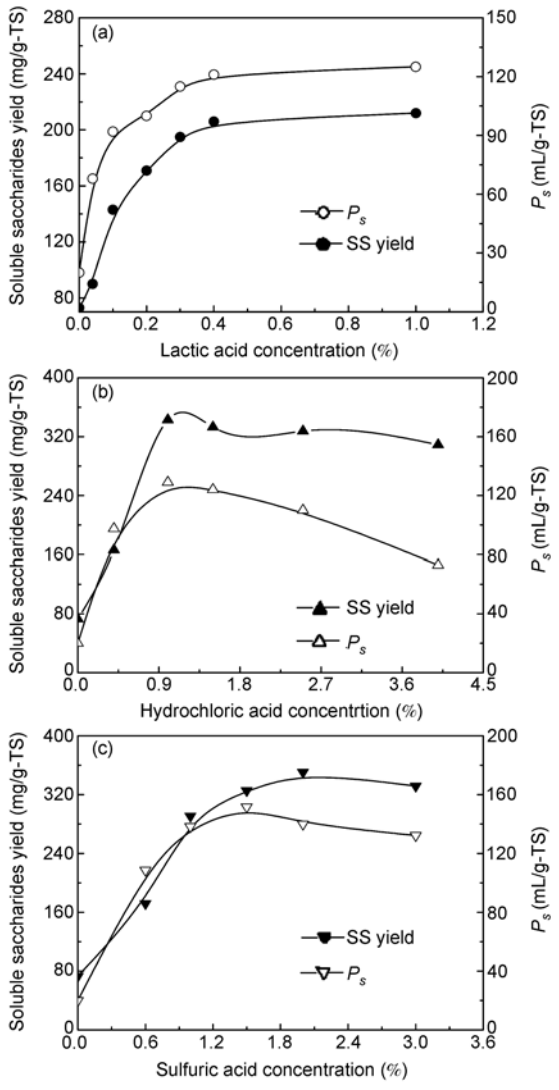

Figure 1 Effect of pretreatment methods on saccharification efficiency and hydrogen yield.

The effects of acid pretreatment on the H_2 yield are presented in Figure 1. A similar behavior for the H_2 yield was observed with the changes in the lactic acid, HCl or H_2SO_4 concentration, the maximum H_2 yield (125 mL/g-TS) by the lactic acid pretreatment appeared at lactic acid 0.4%. Whereas the H_2 yield by the HCl or H_2SO_4 pretreatment increased gradually with the increase of HCl concentration from 0.6%—1.0% or H_2SO_4 concentration from 0.6%—1.5%. Maximum H_2 yield of 129 mL/g-TS at HCl concentration of 1.0%, 151 mL/g-TS at H_2SO_4 concentration of 1.5% was observed, respectively. Thereafter, with further increase of HCl or H_2SO_4 concentration in the pretreatment, the trend was reversed (73 mL/g-TS at 4.0% HCl; 132.2 mL/g-TS at 3.0% H_2SO_4,), respectively. The results are consistent with our previous studies, in which higher anion concentrations of Cl^- and SO_4^{2-} inhibited heavily the growth of the hydrogen-producing bacteria and led to the decrease of bio-hydrogen production capacity. Simultaneously, the bio-hydrogen production systems had to neutralise to pH 7.0 by NaOH, which would result in the accumulation of Na^+ anion and the increase in the osmotic pressure of cell. It also would inhibit the growth of hydrogen-producing bacteria. Herein, the inhibition action of Cl^- anion is stronger than that of SO_4^{2-} anion.

(ii) Acid-enzyme coupling pretreatment. Table 3 summarizes the effects of anaerobic and aerobic atmosphere on saccharification efficiency and hydrogen yield. As shown in Table 3, the anaerobic atmosphere is beneficial to the enzymatic hydrolysis of corn stalk and the hydrogen production, in which the substrate were firstly pretreated by 1.5% H_2SO_4. The yields of SS (400 mg/g-TS) and H_2 (153 mL/g-TS) under anaerobic condition were higher than the data (330 mg/g-TS, 140 mL/g-TS) under the aerobic condition, respectively.

Table 3 The effects of anaerobic and aerobic atmosphere on saccharification efficiency and hydrogen yield

Atmosphere	Dosage (IU/g)	Time (h)	Temperature (℃)	pH	SS yield (mg/g-TS)	P_s (mL/g-TS)
aerobic	11	72	50	4.8	330	140
anaerobic	11	72	50	4.8	400	153

Table 4 shows the SS yield and the estimated kinetic parameters λ, R'_m and P_s at initial pH 7.0 and 36℃. As shown in Table 4, the yields of SS and H_2 increased rapidly with the increase in the enzyme dosage from 348 mg/g-TS and 134 mL/g-TS at 1.1 IU/g to 468 mg/g-TS and 165 mL/g-TS at 17.6 IU/g, respectively, and then leveled off from 22 IU/g to 26.4 IU/g, which is consistent with enzymic kinetics[18,19].

Table 4 Effect of enzyme dosage on saccharification efficiency and hydrogen yield of corn stalk

Dosage (IU/g)	SS yield (mg/g-TS)	P_s (mL/g-TS)	λ (h)	R^2
1.1	348	134	6	0.9864
2.2	348	134.3	5.5	0.9793
4.4	352	135	3.6	0.9899
6.6	364	136.8	1.4	0.9921
11	374	144	0.5	0.9937
17.6	468	165	3	0.9864
22	470	165	3	0.9788
26.4	470	165	4.5	0.9653

(iii) Solid bio-pretreatment. Although the method of solid bio-pretreatment has been widely used for feed processing, no information is available on the bio-hydrogen production using this method. In this section, the feasibility of cellulosse-hydrogen production from the solid bio-pretreated corn stalk was investigated. The results indicate that the process of the bio-pretreatment was accompanied by the generation of lactic acid. Figure 2 illustrates the effects of microbe additive loading versus yields of SS and lactic acid in bio-pretreated corn stalk. It is obvious that an increase of the SS yield could be obtained within the range of 2.5 g/kg to 7.5 g/kg, the SS yield reached the maximum of 212 mg/g-TS at 7.5g/kg, and with further increase in microbe additive dosage the trend was reversed. Yet the lactic acid yield kept an upward tendency in the range of 2.5 g/kg to 12.5 g/kg, in which the maximum yield of lactic acid occurred at the dosage of 12.5 g/kg. This phenomenon was mainly due to the bioconversion of SS into lactic acid.

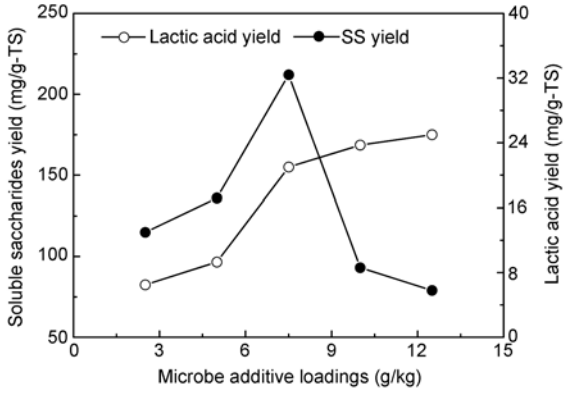

Figure 2 Effect of microbe additive loading on saccharification efficiency and hydrogen yield at 7.5 g/kg corn stalk.

Figure 3 and Table 5 show the data of cellulose-hydrogen production from the bio-pretreated corn stalk. As expected, a significant increase in the H_2 yield could be achieved by raising microbe additive dosage from 2.5 g/kg to 7.5 g/kg. Figure 3 reveals that the plot based on eq. (2) fit the cumulative H_2 production data satisfactorily for bio-pretreated corn stalk at dosage 7.5 g/kg. The maximum P_s of 175 mL/g-TS and R'_m of 14.2 mL/g-TS$\cdot$h^{-1} were observed at the fermentation time of 30h and 10 h, respectively (Figure 3 and Table 5). After the hydrogen fermentation, no SS and lactic acid were detected in the effluent. It indicates that the two kinds of substrates were completely utilized in the process of biohydrogen production.

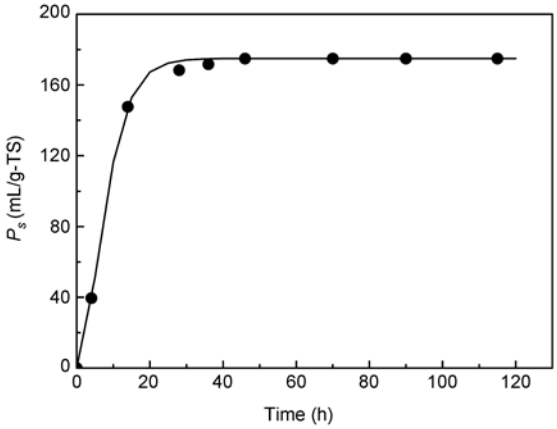

Figure 3 Changes of hydrogen yield with culture time at fixed microbe additive loading of 7.5 g/kg corn stalk.

Table 5 Kinetic parameters of hydrogen production process of corn stalk in the condition of different microbe additive loading

Dosage (g/kg)	λ (h)	R'_m /(mL/(g-TS$\cdot$h))	P_s (mL/g-TS)	R^2
2.5	5.5	11.7	123.5	0.999
5.0	0	8.1	127	0.999
7.5	1.4	14.2	175	0.999
10.0	3.6	8.6	113.5	0.990
12.5	4.5	4.8	81	0.986

To explain the mechanism of cellulose-hydrogen production from bio-pretreated corn stalk, theoretical calculation of H_2 yield was further investigated. Assuming that the enhanced H_2 yield might be only attributed to the generated SS and lactic acid in the bio-pretreated substrate, the estimated H_2 yield should be 63.8 ml/g-TS by butyrate-type fermentation (2 moL H_2/mol glucose) or 127.6 mL/g-TS by acetate-type fermentation (4 moL H_2/ mol glucose)[20]. As can be seen from the above, the experimental result was significantly higher than the calculated values. In addition, the component analyses of the solid bio-pretreated corn stalk and the fermentation producing H_2 residue show that the contents of

hemi-cellulose and cellulose decreased 10.31% and 4.87% in the batch tests, respectively. These results imply that the enhanced H_2 yield related to the direct biodegradation of the hemi-cellulose and cellulose besides the contribution of the generated SS and lactic acid in the bio-pretreated corn stalk during the bio-hydrogen fermentation. The presumption was consistent with the conclusion in section 2.1.

From the above it can be seen that the solid bio-pretreatment was simpler and more effective than the methods of acid pretreatment and acid-enzyme coupling pretreatment, the H_2 yield (175 mL/g-TS) was higher than that in the previous studies. For instance, Datar et al. reported that molar yields of 2.84 (51.7 mL/g) and 3.0 (71.7 mL/g) were obtained using the hydrolyzate of corn stalk derived from neutral and acidic steam explosions, respectively, which indicated a low level of total sugar yield (e.g., 156.8 mg/g from neutral pretreated corn stover, and 204.5 mg/g from acidic pretreated corn stover)[6]. The results show that both the pretreatment of substrate and the H_2-producing microflora played a vital role in the effective conversion of corn stalk into cellulose-hydrogen by mixed culture.

2.3 Verification experiments

The verification tests were performed in a 5 L continuously stirred anaerobic bioreactor (CSABR) (Figure 4), which was filled with 3 L mixture comprising the bio-pretreated corn stalk (15 g/L) and the pre-incubated inoculums (3 L) at fixed pH 5.5, 36℃, HRT 10 h, and 120 $r \cdot min^{-1}$).

(i) Bio-hydrogen production. Figure 5(a) demonstrates the developments of H_2 yield and ORP with the culture time. As shown in Figure 5(a), hydrogen production began immediately after a short lag period. A significant increase of hydrogen yield was observed at the culture time from 0 h to 6 h, and the maximal H_2 production rate of 14.5 mL/g-TS$\cdot h^{-1}$ was obtained at 6 h. The maximal H_2 yield of 175.6 mL/g-TS and H_2 content of 57.2% occurred after about 30 h fermentation. The results of data analysis show that the calculated data (P_s 176 mL/g-TS; R'_m 14.5 mL/g-TS$\cdot h^{-1}$, and λ 0 h) were close to the experimental values with a high value of coefficient of determination (R^2 0.997). It indicated the high degree of precision and good deal of reliability of the experimental values.

(ii) ORP value and biodegradation characteristics of the substrates. The changes of ORP value along with the culture time during the bio-hydrogen fermentation are presented in Figure 5(a). As it is shown in Figure 5(a), the ORP value gradually dropped from −102 mV to −114 mV during the first 4 h, and then reduced rapidly to −446.5 mV in the following 3.5 h. It shows that the reactor was shifted from facultative anaerobic running conditions to strict anaerobic running conditions with the exhaustion of trace oxygen. Thereafter, the ORP value stayed in the range of −445 mV to −455 mV, which was consistent with that in previous reports[21,22].

Usually, ORP value reflects the amount and type of oxidative-reductive substrates in the reactor. The H_2-producing bacteria in the lesser panda manure is a strict

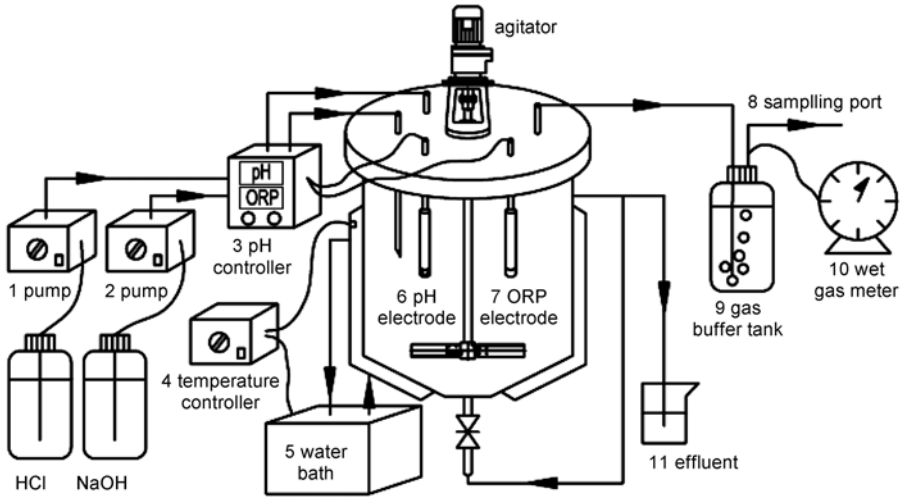

Figure 4 Schematic diagram of the 5 L CSABR.

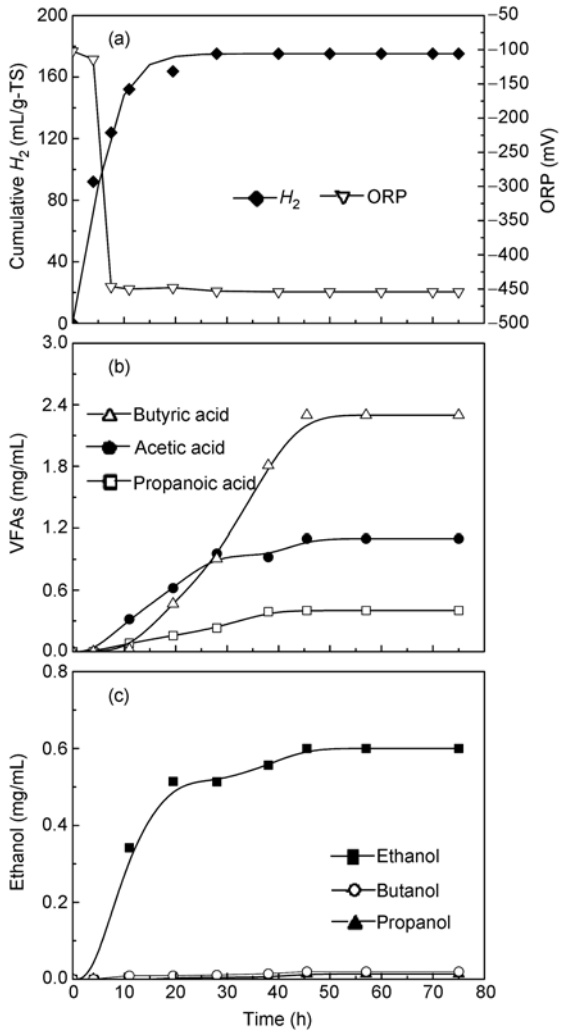

Figure 5 Time-course profile of hydrogen fermentation under optimized conditions from corn stalk in 5 L CSABR.

anaerobe (e.g., *Clostridium sp.*), and exhibits a relative low ORP level in the reactor. It was close to that in some previous reports, e.g., Ren et al. reported the ORP level of –300—–100 mV by the propionic acid type of fermentation and –450 mV to –300 mV by type fermentations of butyric acid and acetic acid treating molasses wastewater by mixed culture, respectively[23].

Considering that bio-hydrogen production was usually accompanied with the key VFA/alcohols produced, here, butyric acid, acetic acid, and alcohol were selected as main metabolic by-products throughout the H_2 producing period. During H_2 fermentation progresses, butyric acid, acetic acid, and ethanol contents reached the maximum yields of 2.3 mg/mL, 1.1 mg/mL and 0.6 mg/mL at about 50 h, respectively, followed by small amounts of butanol and propionate (Figure 5(b) and 5(c)). Among them, the contents of butyric and acetic acids accounted for 70%—80% of the total VFAs. The carbon mass balance was approximately 77.4%, with acetic acid, butyric acid and ethanol as the major carbon by-products along with carbon dioxide. The H_2 percentage in the biogas was 44.3%—57.2% (*v*/*v*) and there was no significant methane observed in the CSABR. The biodegradation characteristics of the substrate were in close agreement with that of earlier reports on H_2 production from sucrose and starch, of which VFAs mainly consisted of acetate and butyrate. The CSABR operated steadily for 170 h with higher H_2 yield and lower H_2 partial pressure level, and the pH value could be easily adjusted by online control system.

3 Conclusions

(1) The lesser panda manure exhibited a good biodegradation characteristic for some carbohydrates, such as glucose, maltose, lactose, xylose, sucrose, and cellobiose. Especially, it could directly convert the microcrystalline cellulose into bio-hydrogen, in which the maximum H_2 yield of 67 mL/g-substrate was observed.

(2) The pretreatment of substrate played a vital role in the effective conversion of corn stalk into cellulose-hydrogen by mixed culture. Among them, the solid bio-pretreatment was the most effective way.

(3) The verification test was performed in a 5 L CSABR. The maximal H_2 production rate and H_2 yield was 14.5 mL/g-TS·h^{-1} and 176 mL/g-TS, respectively.

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